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ARTICLE

THEMATIC SECTION
Consequences of Developmental Exposure to Drugs, Hormones or Altered Environment

CINP

Differential behavioural and neurochemical outcomes from chronic paroxetine treatment in adolescent and adult rats: a model of adverse antidepressant effects in human adolescents?

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Abstract

Selective serotonin reuptake inhibitor use is associated with increased risk of suicidal ideation in adolescent humans, yet the neuropharmacological basis of this phenomenon is unknown. Consequently, we examined the behavioural and neurochemical effects of chronic paroxetine (PRX) treatment in adult and adolescent rats. Rats received PRX in their drinking water (target dose 10 mg/kg) for 22 d, during which time they were assessed for depression- and anxiety-like behaviours. Subsequent *ex-vivo* analyses examined serum PRX concentrations, striatal neurotransmitter content, and regional serotonin and dopamine transporter (SERT, DAT) binding density. After 11–12 d treatment, PRX-treated adolescent rats showed a significant inhibition of social interaction while adults were unaffected. After 19–20 d treatment, adolescents failed to show an antidepressant-like effect of PRX treatment on the forced swim test (FST), while PRX-treated adults showed a typical decrease in immobility and increase in swimming. Two PRX-treated adolescents died unexpectedly after the FST suggesting a compromised response to physical stress. Despite their greater apparent adverse reaction to the drug, adolescents had significantly lower plasma PRX than adults at day 22 of treatment. Chronic PRX treatment had similar effects in adults and adolescents on striatal 5-HT (unchanged relative to controls) and 5-HIAA levels (decreased), while markers of dopaminergic function (DOPAC, HVA, DA turnover) were increased in adults only. SERT density was up-regulated in the amygdala in PRX-treated adolescents only while DAT density in the nucleus accumbens was down-regulated only in PRX-treated adults. These data suggest that the immature rat brain responds differently to PRX and that this might be of use in modelling the atypical response of human adolescents to antidepressants. The age-specific PRX-induced changes in dopaminergic markers and SERT and DAT binding provide clues as to the neural mechanisms underlying adverse PRX effects in adolescent humans.

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Introduction

Selective serotonin reuptake inhibitors (SSRIs) have long dominated the pharmacological treatment of adult depressive disorders (Mant *et al*. 2004). While initially developed for adults, SSRIs have also become a first-line treatment for adolescents with depression (Dean *et al*. 2006). Depressive disorders occur in approximately 4–8% of adolescents (Birmaher *et al*. 1998; Kapornai & Vetro, 2008) and are associated with high rates of self-harm, suicidal ideation and attempts; school dropout; psychosocial, vocational and academic impairment; and persistent depression in adulthood (Birmaher *et al*. 1998; Kapornai & Vetro, 2008; Mann *et al*. 2006; Weissman *et al*. 1999). Fast and

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effective treatment of adolescent depression is therefore of great importance, yet pharmacological approaches are often problematic. Many antidepressants show minimal efficacy in young people (Tsapakis *et al*. 2008; Whittington *et al*. 2004), and SSRIs have been associated with worsening of depression and increased risk of suicidal ideation and behaviour in adolescents (Mann *et al*. 2006; Olfson *et al*. 2006). A black box warning appears on all SSRIs in the USA, notifying prescribers and consumers of this risk (Stone *et al*. 2009). While these effects occur with many SSRIs (Bridge *et al*. 2007; Hammad *et al*. 2006), paroxetine (PRX) is arguably the worst offender (Robertson & Allison, 2009).

The reason for these effects in adolescents is unknown, although several possible causes have been proposed, including non-adherence to medication and subsequent withdrawal symptoms; activating side-effects; and metabolic factors (Caballero & Nahata, 2005). However, few studies have explored potential neurobiological causes. The serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) systems, in particular, are implicated in the causality of depression and suicide, are modified by SSRI treatment, and undergo dramatic reorganization during adolescence, suggesting a possible involvement of these systems in treatment-emergent suicidality.

Depression and suicide have been associated with underactivity of the 5-HT (Mann *et al*. 2001; Ryding *et al*. 2008), noradrenaline (NA) (Brunello *et al*. 2003) and DA (Yadid & Friedman, 2008) systems and antidepressant drugs are thought to act to restore normal functionality of these systems (Millan, 2004). SSRIs selectively bind the serotonin transporter (SERT), inhibiting 5-HT reuptake and enhancing synaptic 5-HT (Frazer, 1997). However, this acute increase in 5-HT is rapidly attenuated by homeostatic activation of 5-HT_{1A} autoreceptors, which inhibits 5-HT release (Hensler, 2003; Newman *et al*. 2004). Thus, the therapeutic efficacy of SSRIs is delayed 2–4 wk until down-regulation of these, and other, serotonergic receptors occurs (Delago, 2004; Yamauchi *et al*. 2006).

Although SSRIs generally have little affinity for DA receptors or the DA transporter (DAT) (Frazer, 1997), they have both acute and chronic effects on dopaminergic function (Dunlop & Nemeroff, 2007). Specifically, dopaminergic activity appears to be temporarily attenuated by acute SSRI treatment, which may contribute to the delay in therapeutic efficacy (Di Matteo *et al*. 2008; Prisco & Esposito, 1995), and then enhanced by chronic treatment, contributing to the relief of DA-related symptoms of depression such as anhedonia (Di Giovanni *et al*. 2008).

The aforementioned brain changes associated with SSRI treatment have largely been determined from the study of adult animals and human subjects and may not accurately reflect the impact of these drugs on the immature brain. Instead, these drug effects may be modified by interaction with developmental changes occurring during adolescence. Such developmental effects may be explored through the use of adolescent animals. The adolescent period in rats occurs from approximately post-natal day (PND) 28 until PND 55, and is broadly analogous to the corresponding adolescent period in humans (Spear, 2007). Stereotypical adolescent behavioural characteristics in both species include greater risk-taking, novelty-seeking, and social interaction (Crews *et al*. 2007) and elevated susceptibility to stress (McCormick *et al*. 2010). Similarly, adolescence in both species is a period characterized by neural maturation and reorganization (Paus, 2005), increases in neurogenesis and synaptic pruning (Hodes *et al*. 2009; Paus, 2005) and changes in serotonergic (Spear, 2000) and dopaminergic (Crews *et al*. 2007) functionality. Compared to adult animals, adolescents have higher synaptic 5-HT and elevated 5-HT receptor and SERT density (Murrin *et al*. 2007). Striatal DA receptors, DAT and DA turnover are also elevated in early adolescence and decline gradually to adult levels during maturation (Crews *et al*. 2007).

In order to explore developmental differences in antidepressant response, the present study investigated differential changes in anxiety- and depression-like behaviours during chronic PRX administration in adult (PND 70) and adolescent (PND 28) rats, and associated developmental differences in plasma PRX concentration, SERT and DAT density and striatal monoamines.

Method

Subjects

The subjects were 32 adolescent (PND 28, 77–118 g) and 32 adult (PND 70, 313–447 g) experimentally naive male albino Wistar rats (Animal Resource Centre, Australia). Rats were housed in age- and treatment-matched groups (four adults or eight adolescents per cage) with food and water freely available, except for rats given PRX for whom water was replaced with PRX solution. The colony room was maintained at 22 °C on a 12-h reversed light/dark cycle (lights on 21:00 hours), with all behavioural testing conducted during the dark phase. All experimentation was approved by the University of Sydney Animal Ethics Committee in accordance with the

Table 1. Summary of experimental procedures

Experiment	5 mg/kg			10 mg/kg																		No drug				
day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Emergence									✓																		
SI											✓	✓															
NSD													✓														
Forced swim[a]																			✓	✓							
Euthanasia 1																						✓					
Washout[a]																							✓	✓	✓	✓	✓
Euthanasia 2[a]																											✓

SI, Social interaction; NSD, novelty-suppressed drinking.
Adult (PD 70–91, $n=32$) and adolescent (PD 28–49, $n=32$) rats received paroxetine (PRX) solution or normal drinking water for 22 d. All rats were exposed to the emergence test, social interaction test, and novelty-suppressed drinking test. Half of the rats ($n=8$/group, randomly selected) were exposed to the forced swim test (cohort 1). The remaining rats (cohort 2) were euthanized on day 22 in preparation for *ex-vivo* analyses. Rats from cohort 1 were euthanized on day 27 following a 5-d washout period.
[a] Manipulations experienced only by rats from cohort 1.

Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Drugs and experimental design

Rats were randomly assigned into four groups ($n=16$): Adult/PRX, Adult/Con, Adolescent(Ado)/PRX and Ado/Con, such that half of each developmental cohort received PRX solution in place of standard drinking water. Administration via drinking water has previously been successfully employed in our laboratory (Thompson *et al*. 2004) and was chosen due to the short half-life of PRX (Owens *et al*. 2000). Paroxetine hydrochloride was obtained from Sequoia Research Biochemicals (UK). A target dose of 10 mg/kg was selected on the basis of previous literature showing antidepressant and neurochemical effects of this dose in adult rats (Carlson *et al*. 1996; Sillaber *et al*. 2008), and to approximate therapeutic plasma concentrations in humans (DeVane, 1999). Prior to target dose administration, a half-dose (5 mg/kg) was administered for 3 d in accordance with recommendations for the treatment of children and adolescents with SSRIs (Fleming, 2007). Taking body weight and fluid intake into account, PRX was dissolved in water at the appropriate concentration, adjusted continuously, and administered for 22 d in light-proof bottles in place of standard drinking water. During treatment, fluid intake was recorded daily and body weight every 3 d.

Behavioural tests

A schematic of the experimental procedure is shown in Table 1. Treatment-emergent suicidality is most prevalent during the early stages of antidepressant treatment (Jick *et al*. 2004), and a possible association exists between these symptoms and the short-term anxiogenic effects of SSRIs (Harada *et al*. 2008). In order to assess whether the response to PRX differs between adolescents and adults in these early stages of treatment rats were exposed to a battery of anxiety tests [emergence, social interaction (SI), and novelty-suppressed drinking (NSD) tests] during the second week of the drug administration period. NSD was conducted towards the end of this week, when it was feasible that antidepressant effects may be emerging. A test of antidepressant efficacy, the forced swim test (FST), was conducted at the end of the drug administration period when adolescent rats were aged PND 46–47. This timing was chosen to mimic the time-course of the antidepressant response (Delago, 2004) and to prevent an impact of swim stress on any subsequent behavioural tests.

Emergence test (*day 9*)

The emergence test was conducted in a $120\times120\times60$ cm wooden arena with a black floor, three white walls and one black wall. A $40\times24\times17$ cm black wooden hide box, with a hinged red Perspex lid, was placed centrally against the black wall. Two spotlights with 150 W PAR-78 globes illuminated the arena to produce a bright open-field aversive to rats. Rats were placed inside the hide box at the start of the 5-min testing period. TrackMate v. 1.0 software (Australia) was used for automatic scoring of behaviours, including: (*a*) latency to emerge, (*b*) open-field time, and

(*c*) risk assessment (time spent with part, but not all, of the head/body protruding from the hide box). The arena was cleaned with 50% ethanol after each test session. Order of testing was counterbalanced by treatment group.

Social interaction (*SI*) *test* (*days 11–12*)

The SI test was conducted in a $120 \times 120 \times 60$ cm black wooden arena illuminated by a 40 W red lamp. Age- and treatment-matched pairs of rats of approximately equal body weight, but from different home cages, were placed together in the arena for 10 min. ODlog software (MacroPod software; http://www.macropodsoftware.com) was used for manual scoring of social behaviours, including: (*a*) following/chasing: one rat following the other within a distance of two body-lengths, (*b*) anogenital interactions, (*c*) adjacent interactions: including play-fighting, climbing over/under, and adjacent lying, (*d*) head-to-head interactions, and (*e*) total SI: time spent in all of the above categories. The apparatus was cleaned with 50% ethanol between test sessions.

Novelty-suppressed drinking (*NSD*) (*day 13*)

A novel paradigm, developed in our laboratory, was employed for the NSD test, loosely based on that previously reported (Dulawa & Hen, 2005). The general paradigm examined consumption of a novel palatable beverage under anxiogenic conditions of bright light, white noise and a novel apparatus. This test employed a lickometer apparatus built in our laboratory (Hargreaves & McGregor, 2007), consisting of 16 separate $28 \times 19 \times 18$ cm drinking chambers. Two 5-mm metallic lick tubes, each connected to a 60 ml vertical syringe reservoir, were extended through the back wall and allowed for delivery of 0.07 ± 0.005 ml aliquots of fluid. Rats licked for diluted (33% v/v) Schweppes raspberry-flavoured cordial for 20 min under a fixed-ratio 3 reinforcement schedule. Illumination was achieved using two Par78 150 W floodlights and continuous white noise was generated by a radio tuned off-station ($\sim$90 dB). The number of licks performed by each rat was recorded by custom software written using Labview software (National Instruments, USA). Other behaviours scored included: (*a*) latency to perform 10 licks (this criterion was chosen to control for very short latencies due to isolated investigative licks early in the session, or accidental activation of the lickometer apparatus by the rat upon entry to the chamber), and (*b*) total cordial consumption.

Forced swim test (*days 19–20*)

Rats ($n = 8$/group, randomly selected) were exposed to the modified Porsolt FST as previously described (Detke & Lucki, 1995). Testing was conducted over 2 d, with a 15-min pretest on day 1 and a 5-min test 24 h later. Rats were placed in a clear Perspex cylinder (46 cm high, 22 cm diameter) containing water (24 ± 1 °C) to a depth of 30 cm. The room was illuminated with a 40 W red lamp, and miniature video cameras relayed images to a video recorder and computer in an adjacent room. An observer, blind to group assignment, manually scored behaviours during the 5-min test session using ODlog software. Scoring was conducted by dividing the test period into 5-s intervals and recording the dominant behaviour (swimming, climbing, immobility) during each interval, as recommended for detection of SSRI-induced behavioural changes (Cryan *et al*. 2002). Tubes were cleaned and refilled with fresh water between trials. After completion of the session, rats were immediately removed from the chambers, gently towelled and placed in a cage for drying.

Neurochemical analysis

On day 22 of drug administration, the 32 rats ($n = 8$/group) that did not experience the FST were euthanized by decapitation. Brains were removed and the left and right striatum manually dissected out over ice, snap-frozen in liquid nitrogen and stored at -80 °C. Samples were then processed for biogenic amine content by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously (Clemens *et al*. 2005) with slight modification.

Biogenic amines were separated using a Phenomenex Synergi Polar-RP 80A (250×4.6 mm) reversed-phase column coupled with a 1-mm Optiguard C_{18} pre-column maintained at 30 °C. The mobile phase consisted of 0.1 M phosphate buffer (pH 3.0), 0.74 mM PIC B-8 octane sulfonic acid (Waters, Australia), 0.3 mM sodium EDTA and methanol (18% v/v). The flow rate was 0.7 ml/min.

SERT and DAT autoradiography

From day 22 of drug administration the remaining PRX-treated rats ($n = 8$/group) underwent drug washout with standard drinking water for 5 d. Drug washout was conducted prior to SERT/DAT autoradiography due to possible competition between the radioligand used and exogenous PRX remaining in the animal (Hirano *et al*. 2005). Five days was deemed

appropriate, with animals being drug free for 15 half-lives of PRX (Owens *et al*. 2000).

Subsequently, rats were euthanized by decapitation. Trunk blood was collected and brains removed, snap-frozen in liquid nitrogen, and stored at −80 °C. Coronal sections (20 μm) were cut on a Microm cryostat, and mounted onto polysine-coated slides (LabServ, Australia). [^{125}I]RTI-55 (Perkin Elmer, USA) was used to assess binding density for SERT and DAT as described previously (Kindlundh-Hogberg *et al*. 2007). SERT binding was determined at the level of the hippocampus and central amygdala (Bregma −2.7 mm), while DAT was assessed within the striatum (Bregma +1.3 mm).

Slides were apposed for 4 d to Kodak Biomax MR film in the presence of standard ^{14}C microscales (American Radiochemical Company, USA). Autoradiographs were developed using Kodak developer and fixer. Films were scanned using a Bio-Rad GS-800 calibrated densitometer and analysed using ImageJ software (NIH, USA). Standard curves were calculated for conversion of optical density to nCi/mg radioactivity concentration, using calibrated microscales.

Serum PRX determination

Trunk blood samples were collected in pre-chilled lithium-heparin blood tubes immediately following decapitation. Serum was separated from cells by centrifugation (3300 *g*, 4 °C, 15 min) and PRX content determined using a solid-phase extraction (SPE) column and HPLC with ultraviolet detection as described previously (Li *et al*. 2004) with slight modification.

An SPE system, consisting of SPEC 3ML MP3 (15 mg) microcolumns (Varian, Australia) connected to a Vac Elut vacuum manifold, was used for sample extraction. Five μl of the resulting solution was then automatically injected into the previously described HPLC system. PRX and the internal standard, clomipramine, were separated using a Waters Symmetry C_8 5-μm (2.1 × 150 mm) microbore reverse-phase column coupled with a 1-mm Optiguard C_8 pre-column. A Shimadzu SPD-M10Avp diode array detector (Kyoto, Japan) monitored at 295 nm was used for quantification.

Statistical analysis

Data were analysed by between-subjects or mixed-model ANOVA followed by planned contrasts comparing (*a*) Ado PRX *vs*. Con, and (*b*) Adult PRX *vs*. Con. Analyses were conducted using SPSS 15 for Windows (SPSS Inc., USA) with significance levels set at 0.05.

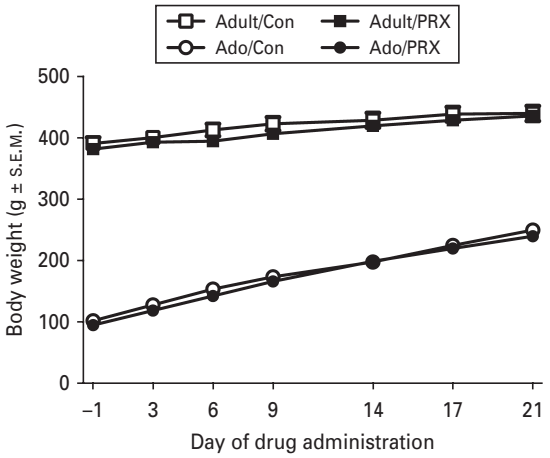

Fig. 1. Effects of 22 d paroxetine (PRX, 10 mg/kg) administration on body weight of adult and adolescent (Ado) rats ($n = 16$/group). Data are presented as average body weight (g) over treatment (mean ± S.E.M.).

Results

Behavioural effects

Body weight

Mixed-model ANOVA revealed no overall effects of PRX on body weight throughout the treatment period (main effect: $p > 0.05$; Fig. 1). As expected, adult rats weighed more than adolescents [$F(1, 60) = 1238.01$, $p < 0.001$], and the main effects were modulated by significant effects of day of drug administration [day main effect: $F(6, 360) = 1684.63$, $p < 0.001$; day × age interaction: $F(6, 360) = 371.09$, $p < 0.001$; day × treatment interaction: $F(6, 360) = 4.16$, $p < 0.001$; day × age × treatment interaction: $F(6, 360) = 2.86$, $p < 0.05$]. Further comparisons investigating these interactions revealed no differences in body weight between treatment groups of the same age either before or during PRX administration, except on day 6 when PRX-treated adults weighed less than age-matched controls [$F(1, 60) = 4.26$, $p < 0.05$].

Fluid intake

Overall, PRX-treated rats consumed less fluid than controls [$F(1, 8) = 71.94$, $p < 0.001$]. Although adults consumed more than adolescents [$F(1, 8) = 45.73$, $p < 0.001$], the reduction in intake due to PRX was greater for adult than adolescent rats [$F(1, 8) = 14.08$, $p < 0.01$]. Further comparisons revealed that intake was significantly higher in Adult/Con than Adult/PRX rats at all time-points following the introduction of PRX ($p < 0.05$) although no difference in intake was present prior to PRX administration. In adolescents,

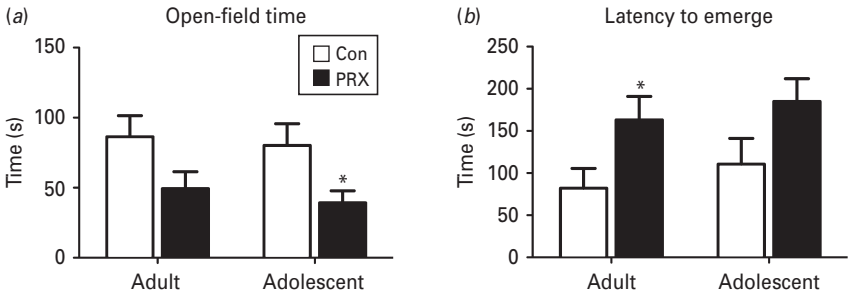

Fig. 2. Effects of paroxetine (PRX, 10 mg/kg) on anxiety-like behaviours in the emergence test (5-min duration, conducted on day 9 of the drug administration period). (*a*) Time spent in the open field, (*b*) latency to emerge from the hide box into the open field. Data are presented as time (s) in the open field and latency (s) to emerge (mean ± S.E.M.). * Significantly different from age-matched controls ($p < 0.05$).

fluid intake did not differ between treated and untreated groups before or during PRX administration, except on day 21 when PRX reduced intake [$F(1, 8) = 6.93$, $p < 0.05$]. Averaged over all days, Adult/Con rats consumed 47.33 ± 2.42 ml/rat.d, which was significantly more than Adult/PRX rats (28.00 ± .31 ml/rat.d), [$F(1, 8) = 82.66$, $p < 0.001$]. In contrast, Ado/Con rats consumed 27.97 ± .44 ml/rat.d, which did not differ from the 22.46 ± 0.39 ml consumed by Adolescent/PRX rats.

Emergence test

Overall, PRX was anxiogenic on the emergence test, decreasing open-field time [$F(1, 60) = 8.75$, $p < 0.01$; Fig. 2*a*] and increasing latency to emerge [$F(1, 60) = 8.05$, $p < 0.01$; Fig. 2*b*]. PRX treatment reduced open-field time in adolescents [$F(1, 60) = 4.84$, $p < 0.05$] and increased latency to emerge in adults [$F(1, 60) = 4.38$, $p < 0.05$]. No differences in risk assessment time were detected in either age group as a result of PRX administration.

Social interaction

Overall, PRX-treated rats spent less time in SI than control rats [$F(1, 28) = 9.76$, $p < 0.01$; Fig. 3*a*]. In adolescents, PRX significantly reduced total SI [$F(1, 28) = 20.40$, $p < 0.001$; Fig. 3*a*], following [$F(1, 28) = 7.90$, $p < 0.01$; Fig. 3*b*], adjacent interactions [$F(1, 28) = 30.34$, $p < 0.001$; Fig. 3*c*] and head-to-head interactions [$F(1, 28) = 9.51$, $p < 0.01$; Fig. 3*d*]. Conversely, there was no effect of PRX on adult social behaviours, or on anogenital interactions in either age group.

Novelty-suppressed drinking

PRX was anxiogenic on the NSD test, increasing latency to reach criterion (10 licks) [$F(1, 60) = 4.19$, $p < 0.05$; Fig. 4*a*]. These anxiogenic effects were somewhat more pronounced in adolescents, who displayed a greater inhibition of consumption in response to PRX than adults [interaction: $F(1, 60) = 6.15$, $p < 0.05$; Fig. 4*b*]. However, overall, neither age nor treatment altered overall consumption. There were no age or interaction effects on latency to criterion.

Forced swim test

Data from one Adult/PRX rat was lost due to computer malfunction. An antidepressant effect of PRX was apparent in adult rats, with increased swimming [$F(1, 25) = 4.39$, $p < 0.05$; Fig. 5*a*] and decreased immobility [$F(1, 25) = 5.04$, $p < 0.05$; Fig. 5*b*] in PRX-treated compared to control adults. PRX had no effect on climbing in adults. Conversely, there were no differences between control and PRX-treated adolescents on swimming, climbing or immobility. In addition, the FST was associated with two unexpected fatalities in adolescents treated with PRX, with one rat found deceased immediately following the pre-test swim, and the other discovered deceased in the home cage the following morning.

Neurochemical analysis

Results of neurochemical analysis are presented in Table 2. Two-way ANOVA revealed a reduction in 5-hydroxyindoleacetic acid (5-HIAA) [$F(1, 28) = 42.89$, $p < 0.001$] and 5-HT turnover [$F(1, 28) = 38.93$, $p < 0.001$] in PRX-treated rats of both ages compared to control rats. There was no overall effect of age on 5-HIAA or 5-HT turnover, or any two-way interaction on either measure. 5-HT was not affected by either variable.

Analysis of dopaminergic markers revealed lower DA [$F(1, 28) = 10.04$, $p < 0.01$], higher DA turnover [$F(1, 28) = 11.43$, $p < 0.01$] and a trend towards lower dihydroxyphenylacetic acid (DOPAC) [$F(1, 28) = 4.01$, $p = 0.055$] in adolescent compared to adult rats. There

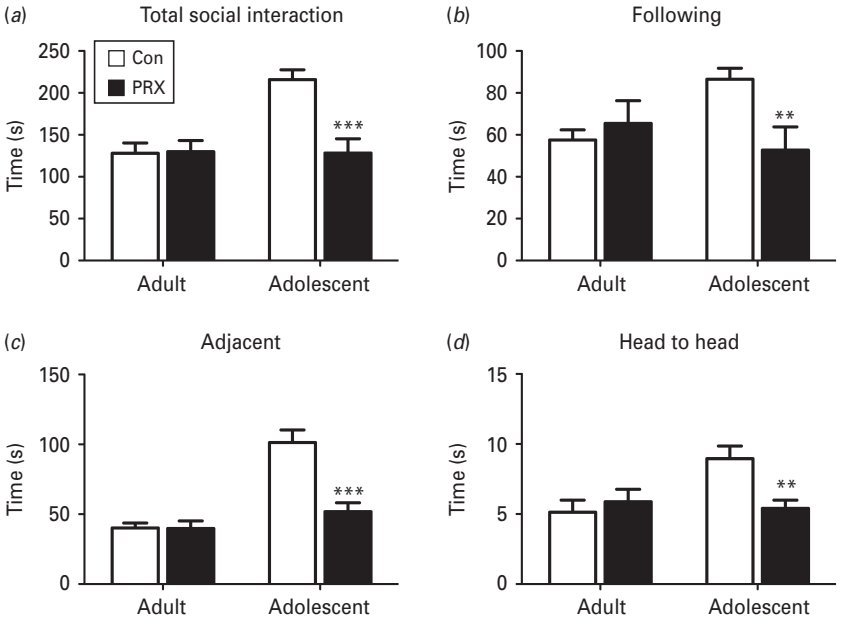

Fig. 3. Paroxetine (PRX, 10 mg/kg) differentially regulates social interaction (days 11–12 of drug administration) in adult and adolescent rats. PRX-treated adolescent ($n=16$/group), but not adult ($n=16$/group), rats show significant reductions in time spent in (*a*) total social interaction, (*b*) following, (*c*) adjacent interactions, and (*d*) head-to-head interactions compared to age-matched controls. Data are presented as total time spent in social behaviours listed (mean ± S.E.M.). Significantly different from age-matched controls: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

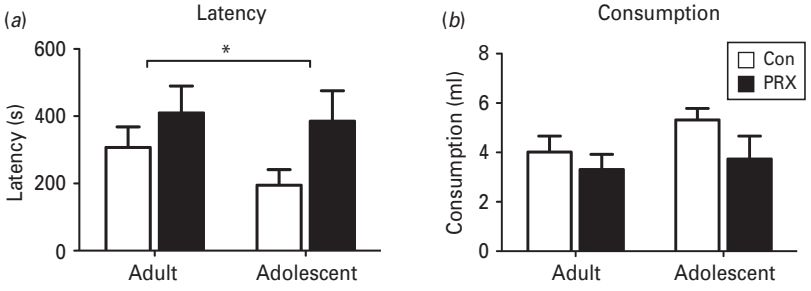

Fig. 4. Effects of paroxetine (PRX, 10 mg/kg) in adult and adolescent rats ($n=16$/group) in the novelty-suppressed drinking test (day 13 of drug administration). (*a*) Latency to perform 10 licks. (*b*) Total cordial consumption (ml). Data are presented as latency (s) and total consumption (ml) (mean ± S.E.M.). * Significant overall treatment effect ($p<0.05$).

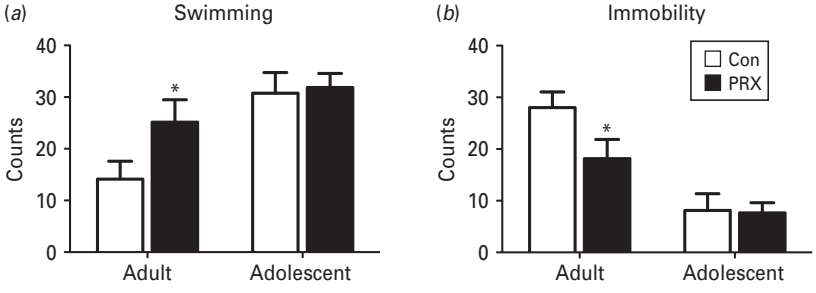

Fig. 5. Exposure to paroxetine (PRX, 10 mg/kg) regulates behaviour on the forced swim test (days 19–20 of drug administration) in adult ($n=8$/group), but not adolescent ($n=6$–8/group) rats. Number of 5-s counts spent with (*a*) swimming, and (*b*) immobility as the dominant behaviour. Data represent mean ± S.E.M. * Significantly different from age-matched controls ($p<0.05$).

Table 2. Neurochemical analysis of striatal monoamine levels and turnover ratios

	Adult		Adolescent	
Measure	Control	Paroxetine	Control	Paroxetine
NA	734.7 (108.3)	787.1 (48.5)	852.4 (81.6)	763.5 (62.5)
5-HT	355.8 (14.5)	343.0 (23.2)	366.9 (30.8)	369.7 (14.3)
5-HIAA	598.1 (60.5)	370.2 (12.3)***	613.9 (30.0)	388.1 (9.5)***
5-HT turnover	1.67 (0.13)	1.11 (0.08)***	1.72 (0.12)	1.05 (0.02)***
DA	8259.3 (455.1)	8359.8 (469.3)	7326.7 (350.9)[a]	6789.4 (271.8)[a]
DOPAC	1785.3 (131.7)	2070.9 (130.7)[b]	1816.8 (143.4)	1548.0 (72.3)[b]
HVA	765.6 (62.4)	968.1 (80.0)*	947.6 (40.3)	827.8 (25.4)
DA turnover	0.09 (<0.01)	0.12 (0.01)**	0.13 (<0.01)	0.12 (<0.01)

NA, Noradrenaline; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT turnover = 5-HIAA/5-HT; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; DA turnover = HVA/DA.
Monoamine concentrations and turnover ratios were analysed over the left and right striatum following euthanasia on day 22 of the drug administration period.
Units of measurement are ng/g wet tissue. Data represent mean (± S.E.M.), $n = 8$/group.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, paroxetine *vs.* control group by age.
[a] Indicates significant overall effect of age ($p < 0.05$).
[b] Indicates significant age x treatment interaction effect ($p < 0.05$).

were no overall effects of treatment on any dopaminergic markers. However, PRX had differential effects in adults and adolescents, as demonstrated by significant interaction effects for DOPAC [$F(1, 28) = 5.10$, $p < 0.05$], homovanillic acid (HVA) [$F(1, 28) = 8.28$, $p < 0.01$] and DA turnover [$F(1, 28) = 6.58$, $p < 0.05$]. Further analysis revealed higher HVA [$F(1, 28) = 6.53$, $p < 0.05$] and DA turnover [$F(1, 28) = 7.75$, $p = 0.01$] in PRX-treated than in control adults, with a numerical increase in DOPAC [$F(1, 28) = 2.71$, $p = 0.11$]. There were no such effects in adolescents. Overall, DA did not vary according to treatment or treatment × age interaction.

There were no age, treatment or interaction effects for NA.

SERT and DAT autoradiography

There were age-specific effects of PRX on SERT density in the basolateral amygdala (BLA) [interaction: $F(1, 24) = 5.22$, $p < 0.05$], with SERT density increased in PRX-treated adolescents [$F(1, 24) = 5.28$, $p < 0.05$], but not in adult rats (Table 3; Fig. 6). There were no observable effects of drug or developmental stage on SERT binding in the CA3 region of the hippocampus. DAT binding density in the nucleus accumbens (NAc) core was differentially altered by PRX in adult and adolescent rats [interaction: $F(1, 23) = 7.84$, $p = 0.01$], despite the absence of an overall age or treatment effect. Further comparisons revealed a significant decrease in NAc DAT in adults [$F(1, 23) = 6.24$, $p < 0.05$] but not adolescents. There were no effects on DAT in the medial and lateral caudate putamen (CPu).

Plasma PRX levels

Analysis of serum samples from PRX-treated rats (no washout) revealed significantly lower PRX concentrations in adolescents (105.19 ± 17.49 nmol/l) than in adults (308.59 ± 80.52 nmol/l), [$F(1, 14) = 6.09$, $p < 0.05$; Fig. 7]. Samples collected following the 5-d washout showed no detectable PRX.

Discussion

Antidepressant treatment of adolescents with depression is very common (Dean *et al*. 2006), yet many studies report minimal efficacy and increased incidence of suicidal ideation in adolescents treated with SSRIs (Bridge *et al*. 2007; Whittington *et al*. 2004). In agreement with these findings, we report that PRX is associated with no antidepressant efficacy and greater adverse effects in adolescent than in adult rats, despite lower serum PRX concentrations in adolescents. We also provide evidence for age-specific changes in neurochemistry and SERT and DAT binding.

Adult/PRX rats displayed decreased immobility and increased swimming, indicative of a SSRI-induced antidepressant response (Bylund & Reed, 2007). However, there was a conspicuous lack of antidepressant-like effects of chronic PRX in adolescent rats in the FST. This agrees with recent reports of

Table 3. Regional densities of serotonin transporter (SERT) and dopamine transporter (DAT)

	Adult		Adolescent	
Brain region	Control	Paroxetine	Control	Paroxetine
SERT				
Bregma −2.7				
CA3	3.612 (0.17)	4.122 (0.30)	3.372 (0.38)	3.687 (0.17)
BLA	5.123 (0.24)	4.778 (0.24)	4.612 (0.33)	5.573 (0.33)*
DAT				
Bregma +1.3				
NAc core	3.159 (0.10)	2.804 (0.05)*	2.680 (0.15)	2.941 (0.15)
Medial CPu	5.137 (0.10)	5.081 (0.16)	4.732 (0.18)	4.970 (0.25)
Lateral CPu	7.303 (0.22)	7.461 (0.26)	6.728 (0.25)	7.189 (0.24)

CA3, field CA3 of the hippocampus; BLA, basolateral amygdala; NAc, nucleus accumbens; CPu, caudate putamen.
SERT and DAT densities were analysed after 22 d paroxetine treatment, followed by a 120-h drug-free washout period.
Transporter density was determined using [^{125}I]RTI-55 quantitative autoradiography.
Units of measurement are [^{125}I]RTI-55 specific binding (nCi/mg). Data represent mean (±S.E.M.), $n = 5$–8/group.
* $p < 0.05$, paroxetine *vs.* control group by age.

age-specific lack of SSRI efficacy in rodents in the FST and novelty-suppressed feeding (NSF) paradigms (Mason *et al*. 2009; Oh *et al*. 2009). It should, however, be acknowledged in the present study that adolescents showed lower overall levels of immobility and high levels of swimming regardless of drug treatment and this may have precluded detection of an antidepressant response due to a ceiling effect. An antidepressant response of adolescents on the FST may have become apparent with a higher dose of PRX given to adolescents.

It is also important to acknowledge that although adolescents and adults received equivalent doses of PRX, mean plasma levels in adults were almost 3-fold those seen in adolescents. Therefore differential effects of PRX in adolescents in the FST might also reflect the lower systemic PRX levels achieved in adolescents. The cytochrome P450 enzymes 2D6 and 3A4, which are largely responsible for SSRI metabolism, are elevated during childhood and adolescence causing accelerated hepatic metabolism of the parent drug (Caballero & Nahata, 2005). As PRX has no active metabolites (Titier *et al*. 2003), antidepressant efficacy is lost as the drug is broken down. However, despite developmental differences plasma PRX levels in both adolescent and adult rats were well within the therapeutic range for humans (10–600 ng/ml; DeVane, 1999), making it arguable whether the PRX concentration in adolescents was insufficient for therapeutic efficacy.

As the FST is not sensitive to the clinically observed 2–4 wk delay in antidepressant efficacy (Cryan *et al*. 2002), we also examined PRX effects on the NSD – a variant of the popular NSF task – as a measure of efficacy. In contrast to the FST, NSF mimics the time-course of the antidepressant response and is only responsive to chronic antidepressant treatment (Bechtholt *et al*. 2006). We found no evidence for PRX-induced anxiolytic effects in this paradigm on day 13 of drug treatment, finding greater latency to drink a palatable cordial solution in PRX-treated rats of both ages. Despite no age differences in the effect of PRX on latency, the reduction in consumption appeared subtly greater in adolescent rats, suggesting that the anxiogenic effect of PRX may be somewhat more pronounced in adolescents than in adults. Suppression of consumption is unlikely to be attributable to anorexic effects of the drug, since PRX appears to be free of such effects (Frazer, 1997; Keller *et al*. 2001) and we saw no evidence of weight loss in adolescent PRX-treated rats in the current study. Rather, the present results can be argued to be consistent with clinical observations of increased anxiety in many humans during the early stages of SSRI treatment (Goodman *et al*. 2007) and in various animal models of anxiety (Dulawa & Hen, 2005; Koks *et al*. 2001) with anxiolytic effects appearing after 3–4 wk (Bechtholt *et al*. 2006; Oh *et al*. 2009). It is possible that these anxiogenic effects may resolve more quickly in adults than in adolescents; in one study 28 d of fluoxetine administration was anxiogenic in juvenile mice, but anxiolytic in adults (Oh *et al*. 2009).

A more robust demonstration of elevated anxiety in PRX-treated adolescents was apparent in the profound

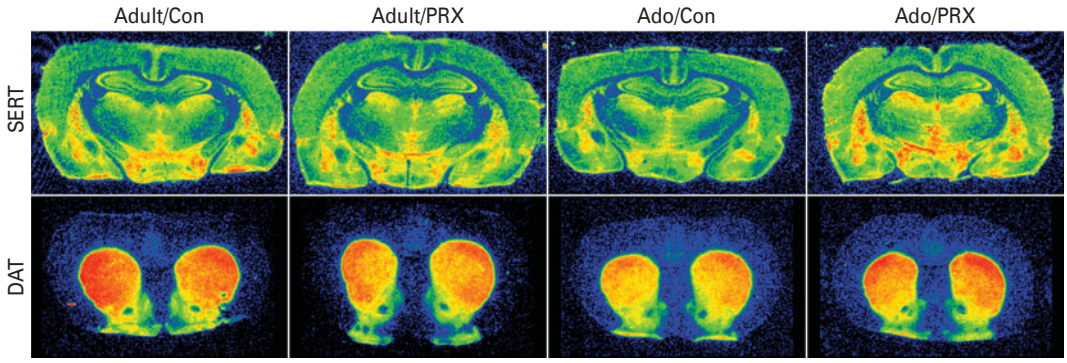

Fig. 6. Representative autoradiograms demonstrating [^{125}I]RTI-55 binding to the serotonin transporter (SERT) and dopamine transporter (DAT). SERT binding density was measured in the CA3 region of the hippocampus and basolateral amygdala (Bregma –2.7), while DAT binding was measured in the medial and lateral caudate putamen and nucleus accumbens core (Bregma (+1.3).

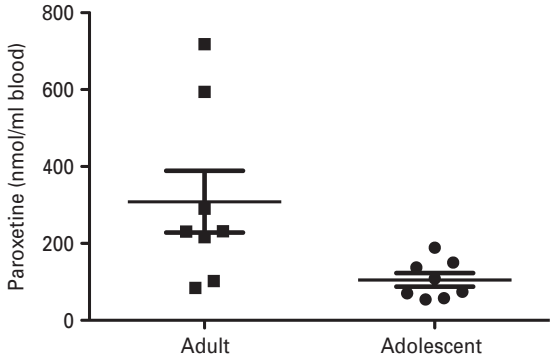

Fig. 7. Paroxetine (PRX) concentrations (nmol/ml) in rat serum ($n = 8$/group) following 22 d PRX (10 mg/kg) administration (no washout).

SI deficits seen in these rats compared to their untreated counterparts. In adolescent male rodents (PND 30–40), play-fighting is the dominant social activity (Pellis & Pellis, 1997). As the SI test was conducted in adolescents at PND 38, the reduction in SI in PRX-treated adolescents probably arose from minimization of play (evidenced by the reduction in adjacent interactions and following/chasing). This is of particular concern given the importance of social play in the development of normal adult sexual behaviour (de Jong *et al*. 2006; Thor & Holloway, 1984). Deficits in social play are key features of childhood depression (Luby, 2009), and have been observed in animal models of genetic susceptibility to depression such as the Wistar Kyoto rat (Malkesman & Weller, 2009). However, it must be acknowledged that baseline social behaviour was greater in adolescent than in adult rats, perhaps providing a greater opportunity to observe inhibitory drug effects. On the other hand, these age-specific inhibitory effects might be seen to be all the more remarkable given the manifestly lower PRX serum levels seen in adolescents compared to adults.

The sudden death of two PRX-treated adolescent rats following the FST pre-test session suggests that PRX may produce a compromised response to physical stress in adolescents. While the precise cause of death is unclear, the potency of PRX at the muscarinic acetylcholinergic receptors (Richelson, 1996) may adversely affect cardiac function. Such potential cardiac effects are underlined by the recently discovered link between PRX use in pregnancy and cardiac malformations in infants (Bar-Oz *et al*. 2007). Future experiments exploring cardiotoxicity during adolescent PRX administration might usefully explore such possibilities.

Subtle, yet possibly important, differences were seen in the neurochemical response to chronic PRX treatment in adult and adolescent rats. There were no age-specific effects of PRX on serotonergic markers, with decreases in 5-HIAA and 5-HT turnover in both adults and adolescents. These effects are well documented in the human and animal literature (Miura *et al*. 2007; Thompson *et al*. 2004) and are thought to result from the SSRI-induced inhibition and/or down-regulation of SERT (Frazer, 1997; Hirano *et al*. 2005), which reduces 5-HT reuptake, preventing intracellular metabolism of 5-HT to 5-HIAA. There were, however, age-specific effects of PRX on the DA system, with general dopaminergic up-regulation in PRX-treated adults and opposite effects in adolescents. SSRI-induced up-regulation of the DA system has been previously reported in adult rodents (Carlson *et al*. 1996; Sekine *et al*. 2007; Tanda *et al*. 1994) and probably occurs indirectly via desensitization/down-regulation of 5-HT$_{2C}$ receptors, which releases the DA system from inhibition (Di Giovanni *et al*. 2008;

Prisco & Esposito, 1995). This may be crucial for the antidepressant response, particularly the relief of symptoms such as anhedonia (Di Giovanni *et al*. 2008; Dunlop & Nemeroff, 2007). The anhedonic consequences of the absence, or partial reversal, of this dopaminergic up-regulation in adolescents may link to the lack of antidepressant efficacy and reduced social interaction (Malkesman & Weller, 2009) observed in the present study.

There were also region-specific changes in SERT and DAT density following PRX administration, with significant up-regulation of SERT in the BLA in adolescent rats, and a tendency towards down-regulation in adults. The minimal effect of PRX on SERT density in adult rats contrasts with the decreases in SERT often reported following chronic SSRI treatment (Benmansour *et al*. 1999; Kovaevi *et al*. 2010), although these effects are by no means universal (Gobbi *et al*. 1997; Hrdina & Vu, 1993) and may be an artefact arising from the short washout periods used in these studies. Competition between the ligand employed for detection of SERT binding and SSRIs necessitates an adequate washout to avoid 'drug on board' effects. In addition, there is some evidence to suggest that the effects of SSRIs on SERT may be short-lived (Hirano *et al*. 2005). The up-regulation in adolescent rats seen in the present study agrees with observations of SERT up-regulation in the frontal cortex of peri-adolescent rats given subchronic fluoxetine (Wegerer *et al*. 1999). This may contribute to adverse behavioural effects: increased membrane SERT would probably increase 5-HT reuptake, reducing synaptic 5-HT and perhaps thereby causing increased anxiety and decreased SI (Gurtman *et al*. 2002).

There is little consistent evidence on effects of SSRIs on DAT density, with some studies suggesting up-regulation of DAT (Kugaya *et al*. 2003), but not others (Chen & Lawrence, 2003). Here we report decreased DAT binding with PRX administration in adult but not adolescent rats in the NAc, a key region of the brain's reward system. The mechanisms underlying these effects are unknown, although it may be a compensatory response to up-regulation of dopaminergic tone in PRX-treated adults mediated by 5-HT/DA interactions via 5-HT_{1A} or 5-HT_{2C} receptor subtypes (Alex & Pehek, 2007). Interestingly, adolescents may not experience the SSRI-induced down-regulation of 5-HT_{2C} receptors experienced in adults (Carrey *et al*. 2002), potentially preventing up-regulation of DA tone and subsequent effects on DAT.

In conclusion, the present study suggests that PRX is less efficacious and associated with more adverse effects in adolescent compared to adult rats. These conclusions are moderated somewhat by the marked baseline differences in behaviour seen in drug-free adolescent rats in the SI test and FST, and also by the more efficient metabolism of PRX seen in adolescents. Future comparisons of adult and adolescent antidepressant responsiveness might also find it difficult to avoid these constitutive developmental differences that cloud the interpretation of adolescent drug effects. Nonetheless, the age-specific alterations in dopaminergic tone and SERT and DAT density in response to PRX give insight into possible causes of the differential behavioural response to PRX reported in our study. In particular, this study points to the possibility that atypical modulation of adolescent dopaminergic and serotonergic systems by SSRIs may underlie some of the adverse reactions to these drugs frequently seen in young persons.

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Statement of Interest

None.

References

Alex K, Pehek E (2007). Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacology & Therapeutics* **113**, 296–320.

Bar-Oz B, Einarson T, Einarson A, Boskovic R, *et al.* (2007). Paroxetine and congenital malformations: meta-analysis and consideration of potential confounding factors. *Clinical Therapeutics* **29**, 918–926.

Bechtholt AJ, Valentino RJ, Lucki I (2006). Identifying neural substrates mediating chronic fluoxetine effects in the novelty-induced hypophagia test. *Neuropsychopharmacology* 31, S94–S95.

Benmansour S, Cecchi M, Morilak DA, Gerhardt GA, *et al.* (1999). Effects of chronic antidepressant treatments on serotonin transporter function, density and mRNA level. *Journal of Neuroscience* **19**, 10494–10501.

Birmaher B, Brent DA, Benson RS (1998). Summary of the practice parameters for the assessment and treatment of children and adolescents with depressive disorders. *Journal of the American Academy of Child and Adolescent Psychiatry* **37**, 1234–1238.

Bridge JA, Iyengar S, Salary BC, Barbe RP, *et al.* (2007). Clinical response and risk for reported suicidal ideation and suicide attempts in pediatric antidepressant

treatment – a meta-analysis of randomized controlled trials. *Journal of the American Medical Association* **297**, 1683–1696.

Brunello N, Blier P, Judd LL, Medlewicz J, *et al.* (2003). Noradrenaline in mood and anxiety disorders: basic and clinical studies. *International Clinical Psychopharmacology* **18**, 191–202.

Bylund DB, Reed AL (2007). Childhood and adolescent depression: why do children and adults respond differently to antidepressant drugs? *Neurochemistry International* **51**, 246–253.

Caballero J, Nahata MC (2005). Selective serotonin-reuptake inhibitors and suicidal ideation and behaviour in children. *American Journal of Health-System Pharmacy* **62**, 864–867.

Carlson JN, Visker KE, Mielsen DM, Keller RW, *et al.* (1996). Chronic antidepressant drug treatment reducing turning behavior and increases dopamine levels in the medial prefrontal cortex. *Brain Research* **707**, 122–126.

Carrey NJ, Dursun S, Clements R, Renton K, *et al.* (2002). Noradrenergic and serotonergic neuroendocrine responses in prepubertal, peripubertal, and postpubertal rats pretreated with desipramine and sertraline. *Journal of the American Academy of Child and Adolescent Psychiatry* **41**, 999–1006.

Chen F, Lawrence AJ (2003). The effects of antidepressant treatment on serotonergic and dopaminergic systems in Fawn-Hooded rats: a quantitative autoradiography study. *Brain Research* **976**, 22–29.

Clemens KJ, Cornish JL, Li KM, Hunt GE, *et al.* (2005). MDMA ('Ecstasy') and methamphetamine combined: order of administration influences hyperthermic and long-term adverse effects in female rats. *Neuropharmacology* **49**, 195–207.

Crews F, He J, Hodge C (2007). Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacology, Biochemistry, and Behavior* **86**, 189–199.

Cryan JF, Markou A, Lucki I (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in Pharmacological Sciences* **23**, 238–245.

de Jong TR, Snaphaan LJAE, Pattij T, Veening JG, *et al.* (2006). Effects of chronic treatment of fluvoxamine and paroxetine during adolescence on serotonin-related behavior in adult male rats. *European Neuropsychopharmacology* **16**, 39–48.

Dean AJ, McDermott BM, Marshall RT (2006). Psychotropic medication utilization in a child and adolescent mental health service. *Journal of Child and Adolescent Psychopharmacology* **16**, 273–285.

Delago PL (2004). How antidepressants help depression: mechanisms of action and clincial response. *Journal of Clinical Psychiatry* **65**, S25–S30.

Detke MJ, Lucki I (1995). Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. *Behavioural Brain Research* **73**, 43–46.

DeVane CL (1999). Metabolism and pharmacokinetics of selective serotonin reuptake inhibitors. *Cellular and Molecular Neurobiology* **19**, 443–466.

Di Giovanni G, Di Matteo V, Pierucci M, Esposito E (2008). Serotonin-dopamine interaction: electrophysiological evidence. *Progress in Brain Research* **172**, 45–71.

Di Matteo V, Di Giovanni G, Pierucci M, Esposito E (2008). Serotonin control of central dopaminergic function: focus on *in vivo* microdialysis studies. *Progress in Brain Research* **172**, 7–44.

Dulawa SC, Hen R (2005). Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neuroscience and Biobehavioral Reviews* **29**, 771–783.

Dunlop BW, Nemeroff CB (2007). The role of dopamine in the pathophysiology of depression. *Archives of General Psychiatry* **64**, 327–337.

Fleming GF (2007). The mental health of adolescents: assessment and management. *Australian Family Physician* **36**, 588–593.

Frazer A (1997). Pharmacology of antidepressants. *Journal of Clinical Psychopharmacology* **17**, 2S–18S.

Gobbi M, Crespi D, Foddi MC, Fracasso C, *et al.* (1997). Effects of chronic treatment with fluoxetine and citalopram on 5-HT uptake, 5-HT1B autoreceptors, 5-HT3 and 5-HT4 receptors in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* **356**, 22–28.

Goodman WK, Murphy TK, Storch EA (2007). Risk of adverse behavioural effects with pediatric use of antidepressants. *Psychopharmacology* **191**, 87–96.

Gurtman CG, Morley KC, Li KM, Hunt GE, *et al.* (2002). Increased anxiety in rats after 3,4-methylenedioxy-methamphetamine: association with serotonin depletion. *European Journal of Pharmacology* **446**, 89–96.

Hammad TA, Laughren T, Racoosin J (2006). Suicidality in pediatric patients treated with antidepressant drugs. *Archives of General Psychiatry* **63**, 332–339.

Harada T, Sakamoto K, Ishigooka J (2008). Incidence and predictors of activation syndrome induced by antidepressants. *Depression and Anxiety* **25**, 1014–1019.

Hargreaves GA, McGregor IS (2007). Topiramate moderately reduces the motivation to consume alcohol and has a marked antidepressant effect in rats. *Alcoholism: Clinical and Experimental Research* **31**, 1900–1907.

Hensler JG (2003). Regulation of 5-HT_{1A} receptor function in brain following agonist or antidepressant administration. *Life Sciences* **72**, 1665–1682.

Hirano K, Seki T, Sakai N, Kato Y, *et al.* (2005). Effects of continuous administration of paroxetine on ligand binding site and expression of serotonin transporter protein in mouse brain. *Brain Research* **1053**, 154–161.

Hodes G, Yang L, Van Kooy J, Santollo J, *et al.* (2009). Prozac during puberty: distinctive effects on neurogenesis as a function of age and sex. *Neuroscience* **163**, 609–617.

Hrdina PD, Vu TB (1993). Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT_2 receptors in rat brain: an autoradiographic study. *Synapse* **14**, 324–331.

Jick H, Kaye JA, Jick SS (2004). Antidepressants and the risk of suicidal behaviours. *Journal of the American Medical Association* **292**, 338–343.

Kapornai K, Vetro A (2008). Depression in children. *Current Opinion in Psychiatry* **21**, 1–7.

Keller MB, Ryan ND, Strober M, Klein RG, *et al.* (2001). Efficacy of paroxetine in the treatment of adolescent major depression: a randomised, controlled trial. *Journal of the American Academy of Child and Adolescent Psychiatry* **40**, 762–772.

Kindlundh-Hogberg AMS, Schioth HB, Svenningsson P (2007). Repeated intermittent MDMA binges reduce DAT density in mice and SERT density in rats in reward regions of the adolescent brain. *Neurotoxicology* **28**, 1158–1169.

Koks S, Beljajev S, Koovit I, Abramov U, *et al.* (2001). 8-OH-DPAT, but not deramciclane, antagonises the anxiogenic-like action of paroxetine in an elevated plus-maze. *Psychopharmacology* **153**, 365–372.

Kovaevi T, Skelin I, Diksic M (2010). Chronic fluoxetine treatment has a larger effect on the density of a serotonin transporter in the Flinders Sensitive Line (FSL) rat model of depression than in normal rats. *Synapse* **64**, 231–240.

Kugaya A, Seneca NM, Snyder PJ, Williams SA, *et al.* (2003). Changes in human *in vivo* serotonin and dopamine transporter availabilities during chronic antidepressant administration. *Neuropsychopharmacology* **28**, 413–420.

Li KM, Thompson MR, McGregor IS (2004). Rapid quantification of fluoxetine and norfluoxetine in serum by micro-disc solid-phase extraction with high-performance liquid chromatography – ultraviolet absorbance detection. *Journal of Chromatography, B: Biomedical Sciences and Applications* **804**, 319–326.

Luby JL (2009). Early childhood depression. *American Journal of Psychiatry* **166**, 974–980.

Malkesman O, Weller A (2009). Two different putative genetic models of childhood depression – A review. *Progress in Neurobiology* **88**, 153–169.

Mann JJ, Brent DA, Argano V (2001). The neurobiology and genetics of suicide and attempted suicide: a focus on the serotonergic system. *Neuropsychopharmacology* **24**, 467–477.

Mann JJ, Emslie GJ, Baldessarini RJ, Beardslee W, *et al.* (2006). ACNP task force report on SSRIs and suicidal behaviour in youth. *Neuropsychopharmacology* **31**, 473–492.

Mant A, Rendle VA, Hall WD, Mitchell PB, *et al.* (2004). Making new choices about antidepressants in Australia: the long view 1975–2002. *Medical Journal of Australia* **181**, S21–S24.

Mason SS, Baker KB, Davis KW, Pogorelov VM, *et al.* (2009). Differential sensitivity to SSRI and tricyclic antidepressants in juvenile and adult mice of three strains. *European Journal of Pharmacology* **602**, 306–315.

McCormick CM, Mathews IZ, Thomas C, Waters P (2010). Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain and Cognition* **72**, 73–85.

Millan MJ (2004). The role of monoamines in the actions of established and 'novel' antidepressant agents: a critical review. *European Journal of Pharmacology* **500**, 371–384.

Miura H, Kitagami T, Ozaki N (2007). Suppressive effect of paroxetine, a selective serotonin reuptake inhibitor, on tetrahydrobiopterin levels and dopamine as well as serotonin turnover in the mesoprefronal cortex of mice. *Synapse* **61**, 698–706.

Murrin LC, Sanders JD, Bylund DB (2007). Comparison of the maturation of the adrenergic and serotonergic neurotransmitter systems in the brain: implications for differential drug effects on juveniles and adults. *Biochemical Pharmacology* **73**, 1225–1236.

Newman ME, Shalom G, Ran A, Gur E, *et al.* (2004). Chronic fluoxetine-induced desensitization of 5-HT1A and 5-HT1B autoreceptors: regional differences and effects of WAY-100635. *European Journal of Pharmacology* **486**, 25–30.

Oh J-E, Zupan B, Gross S, Toth M (2009). Paradoxical anxiogenic response of juvenile mice to fluoxetine. *Neuropsychopharmacology* **34**, 2197–2207.

Olfson M, Marcus SC, Shaffer D (2006). Antidepressant drug therapy and suicide in severely depressed children and adults. *Archives of General Psychiatry* **63**, 865–872.

Owens MJ, Knight DL, Nemeroff CB (2000). Paroxetine binding to the rat norepinephrine transporter *in vivo*. *Biological Psychiatry* **47**, 842–845.

Paus T (2005). Mapping brain maturation and cognitive development through adolescence. *Trends in Cognitive Sciences* **9**, 61–68.

Pellis SM, Pellis VC (1997). The prejuvenile onset of play fighting in laboratory rats (*Rattus norvegicus*). *Developmental Psychobiology* **31**, 193–205.

Prisco S, Esposito E (1995). Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area. *British Journal of Pharmacology* **116**, 1923–1931.

Richelson E (1996). Synaptic effects of antodepressants. *Journal of Clinical Psychopharmacology* **16**, 1S–9S.

Robertson HT, Allison DB (2009). Drugs associated with more suicidal ideations are also associated with more suicide attempts. *PLoS One* **4**, e7312.

Ryding E, Lindstrom M, Traskman-Bendz L (2008). The role of dopamine and serotonin in suicidal behaviour and aggression. *Progress in Brain Research* **172**, 307–315.

Sekine Y, Sukuzi K, Ramachandran PV, Blackburn TP, *et al.* (2007). Acute and repeated administration of fluoxetine, citalopram and paroxetine significantly alters the activity of midrain dopamine neurons in rats: an *in vivo* electrophysiological study. *Synapse* **61**, 72–77.

Sillaber I, Panhuysen M, Henniger MSH, Ohl F, *et al.* (2008). Profiling of behavioural changes and hippocampal gene expression in mice chronically treated with the SSRI paroxetine. *Psychopharmacology* **200**, 557–572.

Spear LP (2000). The adolescent brain and age-related behavioural manifestations. *Neuroscience and Biobehavioural Reviews* **24**, 417–463.

Spear LP (2007). Assessment of adolescent neurotoxicity: rationale and methodological considerations. *Neurotoxicology and teratology* **29**, 1–9.

Stone M, Laughren T, Jones ML, Levenson M, *et al.* (2009). Risk of suicidality in clinical trials of antidepressants in

adults: analysis of proprietary data submitted to US Food and Drug Administration. *British Medical Journal* **339**, b2880.

Tanda G, Carboni E, Frau R, Di Chiara G (1994). Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology* **115**, 285–288.

Thompson MR, Li KM, Clemens KJ, Gurtman CG, *et al.* (2004). Chronic fluoxetine treatment partly attenuates the long-term anxiety and depressive symptoms induced by MDMA ('ecstasy') in rats. *Neuropsychopharmacology* **29**, 694–704.

Thor DH, Holloway WRJ (1984). Social play in juvenile rats: a decade of methodological and experimental research. *Neuroscience and Biobehavioral Reviews* **8**, 455–464.

Titier K, Castaing N, Scotto-Gomez E, Pehourcq F, *et al.* (2003). High-performance liquid chromatographic method with diode array detection for identification and quantification of the eight new antidepressants and five of their active metabolites in plasma after overdose. *Therapeutic Drug Monitoring* **25**, 581–587.

Tsapakis EM, Soldani F, Tondo L, Baldessarini RJ (2008). Efficacy of antidepressants in juvenile depression: meta-analysis. *British Journal of Psychiatry* **193**, 10–17.

Wegerer V, Moll GH, Bagli M, Rothenberger A, *et al.* (1999). Persistently increased density of serotonin transporters in the frontal cortex of rats treated with fluoxetine during early juvenile life. *Journal of Child and Adolescent Psychopharmacology* **9**, 13–24.

Weissman MM, Wolk S, Goldstein RB, Moreau D, *et al.* (1999). Depressed adolescents grown up. *Journal of the American Medical Association* **281**, 1707–1713.

Whittington CJ, Kendall T, Fonagy P, Cottrell D, *et al.* (2004). Selective serotonin reuptake inhibitors in childhood depression: systematic review of published *vs.* unpublished data. *Lancet* **363**, 1341–1345.

Yadid G, Friedman A (2008). Dynamics of the dopaminergic system as a key component to the understanding of depression. *Progress in Brain Research* **172**, 265–286.

Yamauchi M, Miyara T, Matsushima T, Imanishi T (2006). Desensitization of 5-HT_{2A} receptor function by chronic administration of selective serotonin reuptake inhibitors. *Brain Research* **1067**, 164–169.